

There is some evidence suggesting the possibility that chlorophyll may be built up not only from ethyl chlorophyllide and phytyl alcohol, but also from xanthophyll and the products of the photo-oxidation of chlorophyll. The assimilation of carbon dioxide involves a complex series of chemical changes which are reversible in part at least, in which chlorophyll and xanthophyll play a direct chemical part, and in which light acts as an accelerating and possibly as a directive agency.

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*On Forms of Growth Resembling Living Organisms and their Products Slowly Deposited from Metastable Solutions of Inorganic Colloids.*

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[PLATE 1.]

Graham, in his classical papers on colloids, draws attention to the remarkable dynamic properties possessed by matter in the colloidal form, whether as a hydrosol or a hydrogel. He states that "another and eminently characteristic quality of colloids is their mutability. Their existence is a continued metastasis. A colloid may be compared in this respect to water, while existing liquid at a temperature under its usual freezing point, or to a supersaturated saline solution. The colloidal is, in fact, a dynamical state of matter; the crystalloidal being the statical condition. The colloid possesses energia. It may be looked upon as the probable primary source of the force appearing in the phenomena of vitality. To the gradual manner in which colloidal changes take place (for they always demand time as an element), may the characteristic protraction of chemico-organic changes also be referred."\*

It is only within recent years that the importance of these slow metastable variations in colloids so closely simulating the changes in living organisms, which are themselves metastable colloidal complexes, have been appreciated by a few authors, as thus clearly expressed by Graham over 50 years ago.

A metastable colloidal solution is, as Graham states, comparable to a

\* 'Phil. Trans.,' vol. 151, pp. 183-224 (1861).

supercooled solution of a crystalloid, but it differs in that the appearance of the solid or "gel" phase of the colloid does not lead, as in the case of the crystalloidal state, to almost instantaneous separation of the excess of salt and establishment of a stable equilibrium, but instead of that, to a slow drifting towards an equilibrium which may go on for days or weeks or months, and vary in speed, or be actually reversed, with changing characters of environment.

It has been demonstrated by the work of Moore and Roaf\* on the effects of variations in temperature upon the osmotic pressure of gelatine solutions, that a similar metastable condition arises long before any precipitation or passage from a hydrosol to a hydrogel occurs. If a mobile solution of gelatine at a temperature high above the point of gel-formation be raised a few degrees in temperature, the osmotic pressure is considerably increased in excess of the amount demanded by the gas law, showing that there occurs some dissociation in the solution-aggregate of the colloid. If, now, the temperature be allowed to fall back to the original point, only a small drop in osmotic pressure occurs at first and it requires some days before the original level is reached. This peculiar hysteresis requiring a prolonged interval of time for the passage from one condition to another is of great biological interest and may lie at the root of those cyclic alternations, of varying times in different tissues, so characteristic of living matter.

The present communication is concerned with another interesting similarity between inorganic colloids and living structures, namely, that the forms assumed as a result of these slow metastable depositions, or growths, so closely resemble lowly living organisms as to be, in many cases, most difficultly distinguishable from them. In our view, it is this close mimicry between colloidal deposits and living organisms which is responsible for more than one previous observer having described as living organisms such slow growths in metastable solutions.

Rapid osmotic growths between strong solutions of colloids and of crystalloids, capable in most cases of slowly precipitating one another and so forming precipitation membranes, have been studied in most painstaking and ingenious ways by a great host of observers.† These experimentalists have shown that similar effects to production of cell-membranes, skeletons, shells, and tests, and also of mitotic nuclear-division figures, and many

\* 'Biochemical Journ.,' vol. 2, p. 34 (1907).

† Such as Gustav Rose, Runge, Böttger, Traube, Harting, Monnier and Vogt, Quincke, Leduc, Benedikt, Dubois, Herrera, Kuckuck, Albert and Alexandre Mary, and others. Accounts of the literature of the subject are to be found in Leduc, 'Mechanism of Life,' Rebman, London, 1911, and Quincke, 'Annalen der Physik,' 4te Folge, vol. 7, p. 631 (1902).

symmetrical and beautiful forms resembling those of lower aquatic animals may be reproduced by the action of such osmotic energy forms.

The experiments to be recorded in this paper follow much more closely, however, the lines of those devised by Dr. Charlton Bastian,\* and there is an essential difference between the two types.

The forms obtained by the other observers depend upon diffusion effects between highly different solutions placed in close juxtaposition, and so producing steep gradients of variation of concentration with attendant rapid osmotic pressure changes. One of the two solutions is usually a hydrosol as in the Quincke and Leduc experiments, and the other is a fairly concentrated crystalloidal solution or a solid mass of crystals, which reacts with this, producing a precipitation membrane across which the diffusive actions occur. Fluctuations in deposition of this membrane and accidental variations in its thickness and resistance at various points, mainly account for the wonderful forms obtained. Here, doubtless, a great deal of the variations in effect are due to the rate of formation of the hydrogel and its subsequent alterations in properties after formation as degree of aggregation changes.

But in Bastian's type of experiment, there is no formation of a membrane, and no osmotic pressure or diffusion velocity effects arise. The two solutions are thoroughly mixed up from the outset, but in such proportions that the system is just metastable, and so that a deposit is very slowly formed. As will be pointed out later, in describing the details of making up the colloidal solutions, these are just the conditions reached by following the instructions given by Dr. Bastian.

At the outset it is desirable to state that it is our intention to deal only with the peculiar and interesting forms in which growths appear in such metastable solutions of inorganic colloids, and to leave for the moment on one side the larger question as to whether actual living organisms appear in them. The growths we have observed increase in many cases when left in ringed solutions for some days between slide and coverslip. But we may say that we have not been able to obtain experimental evidence that they contain organic carbon compounds, and have not been able to sub-culture them in other media, as has been claimed by Dr. Bastian in regard to his experiments.

The deposits or growths stain with dyes, such as methylene blue, but this, in our opinion, is not evidence that they are organic, for inorganic colloids also adsorb dyestuffs readily and give a staining effect. The growths we

\* See 'The Origin of Life,' by H. Charlton Bastian, M.D., F.R.S., Watts & Co., London, 1911.

have had under examination certainly do not contain cellulose, for they give a negative reply to the iodine and sulphuric acid test for cellulose.

It is now established that many colloidal inorganic substances in presence of sunlight and of water and carbon dioxide can synthesise organic bodies,\* so there is no inherent impossibility that living organisms containing organic carbon compounds can arise from inorganic matter when proper conditions of environment, energy supply, and time are satisfied. But we have been unable to observe anything which we could describe as a living organism arise from these inorganic colloids.

The forms which arise in metastable solutions of inorganic colloids are worthy of consideration as illustrating a mechanism by means of which, when the steps of evolution of the organic from the inorganic have become understood, the study of the origin of the morphology of the microscopic forms of life can find a basis.

At the present moment, and with the lacunæ now existing in our knowledge of the stages intermediate between inorganic evolution and organic evolution, even did undoubted living organisms arise in sterilised and hermetically sealed tubes, their origin would be looked upon with suspicion and ascribed to hypothetical unkilld germs or some chance contamination. The intermediate ground which must be cleared is that of the morphology of inorganic colloids, and the properties of these as catalysts enabling them to build up and synthesise organic compounds. It is from this point of view that we submit a preliminary study of the forms of growths originating in metastable solutions of inorganic colloidal solutions.

The glass tubes used in our experiments were of the same type as those employed by Dr. Bastian in his experiments, and were manufactured for us by the same firm. These tubes are made from glass tubing about 3 cm. in diameter. A rounded bottom of the same diameter as the tube is first blown, then at about 7 cm. from the bottom the tube is drawn off to a tapering point and sealed at the end, the whole length of the tube, including both wide and narrow parts, being about 17 cm.

The tubes were delivered to us sealed, and were first broken when the experiment was to be commenced by filling in the colloidal solutions. It is quite obvious that in the process of manufacture the whole internal surface of the tube would require to be so strongly heated by the glass-blower as to incinerate and destroy utterly any possible organic fibres from entrance of dust or vegetable fibre, and the greatest care was taken by us to prevent ingress of adventitious fibres in any of the subsequent observations and the after manipulations for microscopic observation. All slides and coverslips

\* Moore and Webster, 'Roy. Soc. Proc.' B, vol. 87, p. 163 (1913).

were carefully cleansed with chromic acid and alcohol, and were flamed and examined with the microscope before using them. All pipettes and lifters were also flamed. In the manufacture of the tubes the formation of the rounded bottom of the tube must have heated the glass red-hot for a distance of 3 or 4 cm. at least from the bottom, and the drawing out at the top must similarly have heated all the remainder. We are accordingly quite satisfied that the deposits or growths we are dealing with were actually formed in the tube-contents after the tubes were sealed off and left standing.

Each tube after filling was hermetically sealed and then sterilised by heating in a steam autoclave for 15 minutes at 110° C. The tubes have a capacity of about 100 c.c. and were about one-third filled with the selected mixtures of colloids. The filling was carried out by nipping off at the narrow end, warming the tube, and dipping into the prepared mixture of colloids contained in another sterilised tube (as in the process of filling a thermometer bulb); the tube was then resealed and autoclaved as stated above.

The solutions used were those described by Dr. Bastian as the "yellow" solution and the "colourless" solution, and the same solutions were also employed with the addition of two drops of 5-per-cent. sodium carbonate solution in 30 c.c. of total fluid. This small amount of sodium carbonate was added in order to provide material for formation of organic carbon compounds if there was any tendency towards such a growth of organic matter. In no case, however, have we been able to assure ourselves that there was any formation of organic carbon compounds. We feel certain that there was no appreciable growth of such compounds, but would like to leave this question open because it is so difficult of solution.

At the expiration of nearly seven months some of the tubes were opened and examined microscopically, when the growths shown by the microphotographs (Plate 1) were observed.

Some of the growths observed closely resemble exceedingly fine vegetable fibres, such as filaments of cotton fibre, but finer in structure. Some are rounded like silk fibres, others show flat bands like cotton fibres. Other growths show branched and sometimes transversely divided filaments like hyphæ of moulds. These inorganic growths, indeed, so closely resemble vegetable fibres that we were assured by two competent histologists, to whom we showed them, that they were fibres obtained by leaving cotton filaments on the slides in the process of preparation, and that the use of the reagents for cellulose would certainly demonstrate this fact.

This suggestion was valuable because it showed quite clearly, on application, that the growths were not organic, or, at least, were not cellulose; for

these peculiar deposits do not stain when treated with iodine and sulphuric acid. It is, however, exceedingly difficult, in the technique of microscopic examination, to exclude contamination from the air, and also in some cases the nature of cellulose fibres might be so altered that they no longer gave the typical reactions of cellulose.

*Experimental Procedure.*

The solutions used were made up following as closely as we could the procedure recommended by Dr. Bastian, and in order to do so, we obtained the pharmaceutical solutions from the same manufacturing chemists. The "liquor ferri pernitratis" used was obtained from Messrs. Martindale, and the "sodium silicate" (sp. gr. 1.44) from Messrs. Allen and Hanbury. Our thanks are due to Dr. Bastian for aiding us in obtaining the same materials, as nearly as possible, which were used for his own experiments. The sodium silicate solution was diluted before use with an equal volume of distilled water as recommended by Dr. Bastian.

"Liquor ferri pernitratis" of the British Pharmacopœia contains 3.3 per cent. of iron, and of this 8 drops, from a dropper giving 29 drops to the cubic centimetre, were added to a total volume of 30 c.c. of distilled water, and a minute amount of sodium silicate, viz., two drops of the sodium silicate (Martindale) diluted one half. The percentage of iron contained in the colloidal solution in which the growths or deposits appeared would accordingly be approximately 0.03 per cent. The amount of sodium silicate is also very minute, and is just sufficient, when instructions are followed, to send both itself and the ferric nitrate into the metastable colloidal condition.

The instructions of Dr. Bastian are to use constantly 8 drops of the iron salt solution to the ounce (about 28 c.c.) of distilled water, and then, according to the varying alkalinity of the sodium silicate solution, to add 2, 3, or 4 drops of it, so as to obtain a mixed solution that is faintly acid or neutral, and which on boiling for 10 minutes yields, after standing for some time, only a very small amount of deposit. Solutions giving no deposits after boiling, or those which, on the other hand, are completely deposited, form no growths. This balance was obtained, in the case of our solutions, when 8 drops of the ferric nitrate solution and 2 drops of the sodium silicate solution were used to 30 c.c. of distilled water; the equilibrium is a very sensitive one.

Now it is to be observed here, that the ferric nitrate is an acid solution readily thrown down by alkali, and the sodium silicate is an alkaline solution from which silicic acid is readily thrown out by acid. When these two solutions are mixed in properly balanced quantities, as is done

in these experiments, there is obtained a mixture in common solution of two metastable colloids, viz., ferric oxide and silicic oxide. These are the favourable conditions for the deposit of the peculiar growths that have been observed.

The above solution, following Dr. Bastian, is called the "yellow" solution. The colourless solution, which probably contained one metastable colloid only, viz., silicic acid, was prepared, following again fairly closely Dr. Bastian's directions. To 30 c.c. of distilled water 2 drops of the sodium silicate solution were added, then 6 drops of "dilute phosphoric acid solution, B.P." (Martindale), and a few crystals of ammonium phosphate (Martindale).

Here, again, an alkaline silicate solution is taken which, with the phosphoric acid solution, would yield at first a metastable colloidal solution of silicic acid, and then with a greater excess a precipitate of silicic acid. The alkaline and acid salts are just so balanced in the above proportions that only a small proportion of the silicic acid is thrown out on autoclaving, or within a short period of a few hours thereafter.

Attention is drawn here to these important points in the chemistry of inorganic colloids, because Dr. Bastian by long and patient experimentation appears to have arrived at the proportions most favourable to the appearance of these growths. To the physical chemist it is obvious that the proportions are just those which will give rise to a metastable solution of a colloid, or a metastable mixture of colloids.

It is under such conditions that slow deposition will take place and cyclic variations can occur, if there be, during a long time interval, up-and-down variations in physical factors of environment. Such are also the conditions in a living cell, and hence the interest in the fact that so many of the appearances shown by the growths are those seen in cell-products.

In October, 1912, between the 18th and 24th, 48 tubes were prepared and autoclaved as above described, the contents of the sets being as shown in the following Table; the qualities of reagent stated were added to 30 c.c. of distilled water in each case.

Thus there were a dozen tubes of each class, viz. one dozen "yellow" solution, one dozen "colourless" solution, one dozen "yellow" solution *plus* sodium carbonate, and one dozen "colourless" solution *plus* sodium carbonate.

It may be said at once that the subsequent examination at a period of seven months afterwards showed no observable difference in the growths, either favouring or deterrent, due to the sodium carbonate.

## Preparation of Tubes.

No. of tube.	Contents "yellow."	No. of tube.	Contents "colourless."
4	2 drops silicate, 8 drops ferric nitrate, 2 drops sodium carbonate.	4a	2 drops silicate, 6 drops phosphoric acid, ammonium phosphate, 2 drops sodium carbonate.
5	" " "	5a	" " "
6	" " "	6a	" " "
October 18, 1912. The 6 tubes were autoclaved for 15 minutes at 115° C. For a few seconds during the period the temperature reached 120° C.			
7	As above, except no sodium carbonate was added.	7a	As above, with omission of sodium carbonate.
8	" " "	8a	" " "
9	" " "	9a	" " "
All heated for 15 minutes in autoclave, temperature 105–115° C., on October 21, 1912.			
10	Same as Nos. 7, 8, and 9. No sodium carbonate.	10a	As Nos. 7a, 8a, and 9a. No sodium carbonate.
11	" " "	11a	" " "
12	" " "	12a	" " "
13	" " "	13a	" " "
14	" " "	14a	" " "
15	" " "	15a	" " "
All 12 tubes autoclaved for 15 minutes at 108–110° C. on October 22, 1912.			
16	As Nos. 4, 5, and 6. Contain sodium carbonate.	16a	As Nos. 4, 5, and 6. Contain sodium carbonate.
17	" " "	17a	" " "
18	" " "	18a	" " "
19	" " "	19a	" " "
20	" " "	20a	" " "
21	" " "	21a	" " "
Heated in autoclave for 15 minutes at 110° C. on October 23, 1912.			
22	As Nos. 7, 8, and 9. No sodium carbonate.	22a	As Nos. 7, 8, and 9. No sodium carbonate.
23	" " "	23a	" " "
24	" " "	24a	" " "
Heated in autoclave for 15 minutes to 112° C. on October 24, 1912.			
25	As Nos. 4, 5, and 6. Contain sodium carbonate.	25a	As Nos. 4, 5, and 6. Contain sodium carbonate.
26	" " "	26a	" " "
27	" " "	27a	" " "
Heated in autoclave for 15 minutes on October 24, 1912.			

On October 29, 1912, the four dozen tubes were disposed as follows:—

West window.	East window.	North window.	South window.	In dark cupboard.	
Nos. 10, 10a	Nos. 12, 12a	Nos. 5, 5a	Nos. 19, 19a	Nos. 4, 4a	Nos. 21, 21a
" 11, 11a	" 22, 22a	" 6, 6a	" 20, 20a	" 18, 18a	" 25, 25a
" 16, 16a	" 26, 26a	" 7, 7a	" 14, 14a	" 9, 9a	" 23, 23a
" 17, 17a	" 27, 27a	" 8, 8a	" 15, 15a	" 13, 13a	" 24, 24a



The examination of the contents of the tubes and the deposits in them under the microscope were commenced on May 29, 1913, that is about seven months after they had been filled and sealed and sterilised.

The peculiar growths shown in some of the appended microphotographs were then seen in all the tubes examined, more abundantly in the "yellow" solution tubes than in those containing "colourless" solution, but still plentiful in these also. The tubes in the windows were richer in growth than those kept in darkness, but the latter did contain growths also, and it would be impossible without more evidence to say whether the greater result in the windows might not be due to greater diurnal fluctuations of temperature than those that take place in the dark cupboard.

The chief appearances observed in the contents of these tubes are:—  
(1) Patches of sometimes fine, sometimes coarse, granular deposits with fine fibres running in them. (2) Chains of dots, sometimes slightly elongated, like micrococci or short bacteria. (3) Branching coarse fibres like hyphæ of a fungus. (4) Coarse fibres, sometimes rounded, sometimes flat and twisted like a cotton fibre; these are very long, and sometimes run more than the whole diameter of a low-power field, sinuous on their course and quite unlike anything crystalline; as mentioned earlier, these may be adventitious. (5) Excessively fine fibrils, also very long and often taking the most fantastic shapes, sometimes they form a network like a fibrin network, at other times they are single and convoluted into the most intricate knots or loops; this type of fibre is finer than any cotton or silk fibre.

All the tubes were examined carefully, but it would serve no purpose to write a detailed description of each, as the appearances were so similar; so the following descriptions may serve as examples:—

*Tube No. 11* (West window, Yellow solution, no soda). Examined May 29, 1913.—This shows plenty of both fine fibres and masses of débris, and small black dots, sometimes in rows like cocci. A very good dip is obtained from supernatant fluid, near wall of tube, away altogether from the coarse deposits, showing very fine branching and interlacing fibres, like minute hyphæ.

*Tube No. 17* (West window, Yellow solution, plus soda). Examined May 29, 1913.—This is very similar to the above and contains both types of fibres.

*Tube No. 26* (East window, Yellow solution, plus soda). Examined May 30, 1913.—Very rich, both in fine, interlacing fibres and in the long, coarse, and twisted fibres. The latter do not give any cellulose reaction with iodine and sulphuric acid. Also, they are yellow hued and evidently contain colloidal iron. Other slides examined give the same results. Many long, delicate fibres often twisted into intricate loop patterns. Only yellow in colour; no iodine staining. The sulphuric acid appears to dissolve out much of the

granular matter resembling protoplasmic débris, and leaves the long fibres and shorter nests of exquisitely fine fibrils more clear.

In the appended microphotographs are shown growths from these and other tubes of the series.

These growths, obtained in hermetically sealed tubes, autoclaved up to 110–116° C. after sealing, and in tubes in which all organic matter must have been completely destroyed in the process of manufacture, appear to us to be proven conclusively not to be caused by any contamination with adventitious organisms. Whatever view may be taken as to the nature of these appearances, our opinion is that they are not adventitious or due to contamination, but that they arise *de novo* in the tubes by a process of growth or deposition from the balanced colloids.

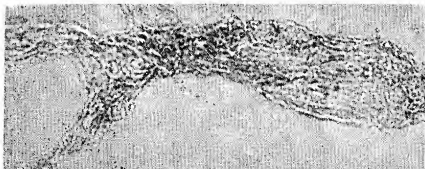
While the boiling or autoclaving was essential in the first stages of the investigation in order to rule out contamination, this process undoubtedly disturbs to a great extent the metastable condition of the colloids and throws out a good deal of the substances, and, in addition, probably interferes with the labile condition of the remainder short of actually precipitating it.

In order to get rid of this inactivation by heat, it was determined, after the growth in autoclaved solutions had once been settled, to experiment with tubes containing metastable colloidal systems which had only been exposed to a moderate degree of heating so as not to disturb any thermo-labile colloids present. The result was that much more growth occurred of all the constituents described.

A series of tubes, filled as described above, were prepared in April, 1914; but, instead of autoclaving, these tubes were heated for 20 minutes to 50° C. and then allowed to stand for two months. At the end of the period, this set of tubes showed very little deposit, but the forms found in the deposit were beautifully developed.

It is to be remembered here that none of the tubes contained any nutrient materials for ordinary micro-organisms, and that heating to 50° C. for 20 minutes would have destroyed any common forms likely to be present. The forms, moreover, closely resembled those found much more sparsely in the autoclaved tubes, and the explanation of the abundance appears to lie in the fact that the colloids were left in a more labile condition.

It is admittedly impossible to exclude completely the objection of contamination in this latter series of experiments. Other methods of causing metastability in mixtures of inorganic colloids and so producing growths more rapidly have been studied and are described in the succeeding paper.



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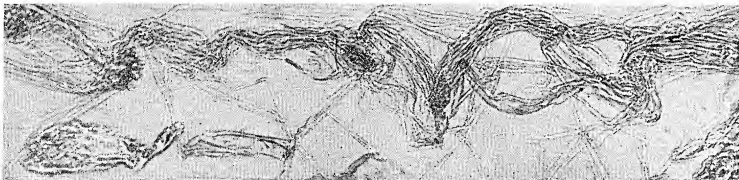
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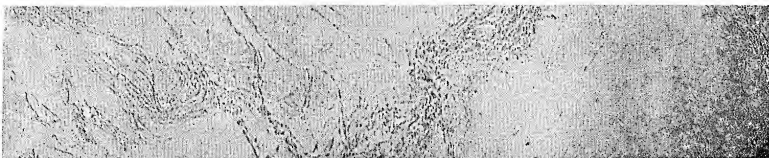
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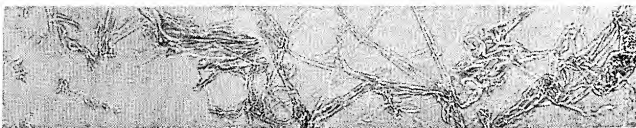
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11



10

DESCRIPTION OF PLATE.

- Fig. 1.—Deposit from Tube No. 8. ( $\times 250$ .) After autoclaving for 15 minutes at 105–115° C.  
Fig. 2.—Deposit from Tube No. 4. ( $\times 250$ .) Autoclaved previously, 105–115° C.  
Fig. 3.—Symmetrically Arranged Deposit from Tube No. 7. ( $\times 250$ .) Not really crystalline. Autoclaved.  
Fig. 4.—Coarse Feathery Deposit from Tube No. 17. ( $\times 250$ .) Autoclaved.  
Fig. 5.—Fine Deposit from Tube No. 5. ( $\times 250$ .) Autoclaved.  
Fig. 6.—Deposit from Tube No. 7. ( $\times 250$ .) Autoclaved.  
Fig. 7.—Hyphæ-like Deposit from Tube No. 20. ( $\times 240$ .) Autoclaved.  
Fig. 8.—Fine Deposit, Tube 20. ( $\times 240$ .) Autoclaved.  
Fig. 9.—Long Looped Fibre from “Colourless” Solution. Metastable silica only. ( $\times 440$ .) Autoclaved.  
Fig. 10.—Mixed Deposit in “Yellow” Solution. Metastable silica and ferric hydrate. ( $\times 240$ .) Autoclaved.  
Fig. 11.—Hyphæ-like Deposit in Yellow Solution. ( $\times 240$ .) Autoclaved.
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*The Production of Growths or Deposits in Metastable Inorganic Hydrosols.*

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(Received February 6, 1915.)

[PLATES 2 AND 3.]

The results described in the preceding paper conducted me to the study of other methods for obtaining these growths.

The fundamental law established by Pasteur, and now universally confirmed, that organic growth cannot occur in sterilised organic media, leaves a curious hiatus between inorganic evolution and organic evolution.

It is a remarkable historical fact that organic evolution was firmly established a full generation before inorganic evolution, and that, with the exception of certain ingenious hypotheses, the theory and facts of inorganic evolution have only been partially ascertained in late years.

The problem presents two distinct yet closely related lines of attack. One concerns the method by which organic compounds can be built up from inorganic sources, and is more purely a question of energy-transformation; the other is related to the morphology, or minute anatomy, in the region lying between the inorganic and the organic, and deals with the colloidal inorganic forms preceding the organic structures. Energy-transformations, although of



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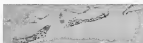
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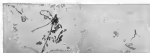
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